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Analysis Of Rev1p And Pol ζ In Mitochondrial Mutagenesis Suggests An Alternative Pathway Of Damage Tolerance

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Abstract

Ultraviolet light is a potent DNA damaging agent that induces bulky lesions in DNA which block the replicative polymerases. In order to ensure continued DNA replication and cell viability, specialized translesion polymerases bypass these lesions at the expense of introducing mutations in the nascent DNA strand. A recent study has shown that the N-terminal sequences of the nuclear translesion polymerases Rev1p and Pol ζ can direct GFP to the mitochondrial compartment of *Saccharomyces cerevisiae*. We have investigated the role of these polymerases in mitochondrial mutagenesis. Our analysis of mitochondrial DNA point mutations, microsatellite instability, and the spectra of mitochondrial mutations indicate that these translesion polymerases function in a less mutagenic pathway in the mitochondrial compartment than they do in the nucleus. Mitochondrial phenotypes resulting from the loss of Rev1p and Pol ζ suggest that although these polymerases are responsible for the majority of mitochondrial frameshift mutations, they do not greatly contribute to mitochondrial DNA point mutations. Analysis of spontaneous mitochondrial DNA point mutations suggests that Pol ζ may play a role in general mitochondrial DNA maintenance. In addition, we observe a 20-fold increase in UV-induced mitochondrial DNA point mutations in *rev* deficient strains. Our data provides evidence for an alternative damage tolerance pathway that is specific to the mitochondrial compartment.

Keywords

mtDNA repair; mutagenesis; translesion synthesis; UV damage; Rev1p; Pol ζ

1. Introduction

DNA is constantly damaged by a variety of endogenous and exogenous sources. Lesions in DNA are converted into mutations via error-prone mechanisms of damage tolerance [1]. Translesion polymerases are specialized enzymes that are required for DNA synthesis across from unrepaired damage. These polymerases are minimally processive and frequently have a specific insertion preference, which may lead to mutations in the newly replicated strand. Since replicative polymerases have stringent requirements for their substrates and cannot accommodate bulky DNA lesions in their active sites, translesion synthesis (TLS) is imperative for lesion bypass. These highly conserved polymerases have been identified in organisms from bacteria to humans. The consequence of error-prone TLS is demonstrated by mutations in error-free pathways that lead to human diseases, including skin cancers and a variant form of xeroderma pigmentosum (XP-V) caused by the absence of a functional non-mutagenic translesion polymerase Pol η [2,3].

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Here we focus on the yeast translesion polymerases Rev1p and Pol ζ , comprised of Rev3p and Rev7p. Genes encoding these proteins were identified in genetic screens for mutants deficient in reversions of specific nuclear mutations (*REVersionless*) [4,5]. These genes are in the *RAD6* epistasis group and mutations in any of these three genes lead to a dramatic decrease in UV-induced mutagenesis in the nuclear genome [4–8]. The sequence of *REV3* revealed homology with DNA polymerase catalytic domains suggesting that it may contain polymerase activity [9]. In 1996, polymerase activity was demonstrated with the addition of the accessory protein Rev7p [10], and the resulting complex was named Pol ζ . This polymerase is able to replicate past UV-induced DNA lesions, although the synthesis is usually mutagenic [11].

Rev1p is a Y-family polymerase that exhibits deoxycytidyl transferase activity, preferentially inserting dCTP across from abasic sites [12]. The pyrimidine dimer bypass activity of Pol ζ is further enhanced by the addition of Rev1p [12]. However, since the deoxycytidyl transferase activity of Rev1p is not essential for UV mutagenesis, the mechanism for its role in Pol ζ -dependent translesion synthesis remains unclear [13].

In 1987, it was shown that a *rev3* mutant strain resulted in a decreased frequency of EMS-induced reversions of a specific mitochondrial mutation [14]. A subsequent study indicated that there was a reduction in the frequency of both spontaneous and UV-induced mitochondrial frameshifts in *rev* mutant strains [15]. However, in spite of the reduction in frameshift mutations in the *rev* mutant strains as compared to wild-type, there was still an induction of mitochondrial frameshifts in response to UV light in the *rev* deficient strains. In addition, the N-termini of Rev1p, Rev3p, and Rev7p could target GFP to the mitochondrial compartment [15].

In this study we analyze the role of Rev1p and Pol ζ in mitochondrial mutagenesis. If these proteins perform a similar function in the mitochondria, as they do in the nucleus, we would expect a decrease in UV-induced mitochondrial DNA mutations in strains lacking the components of this pathway. Here we analyze the effects of deleting the *rev* genes on spontaneous and UV-induced mitochondrial DNA stability and maintenance.

2. Material and methods

2.1. Growth Media

All growth media used in this study are as follows. YPD and YPG medium contained 1% Bacto™ yeast extract (Becton, Dickson and Company), 2% Bacto™ peptone (Becton, Dickson and Company), and 2% dextrose or 2% glycerol, respectively. YPG + 0.1% is YPG medium supplemented with 0.1% dextrose. Synthetic dextrose and synthetic glycerol growth medium contained 0.17% Difco™ yeast nitrogen base (Becton, Dickson and Company), 0.5% ammonium sulfate, the appropriate amino acids as described [16], and 2% dextrose or 2% glycerol, respectively. The final pH of the synthetic growth medium was adjusted to 5.6. The expression of the *kanMX* gene was selected for on media containing 200 mg/L Gibco™ geneticin (Invitrogen). Erythromycin resistant mutants were selected on YG plates containing 50mM sodium phosphate buffer (pH 6.5) and 4 g/L erythromycin (MP Biomedicals, LLC) [17]. Canavanine resistant mutants were selected on synthetic medium lacking arginine and supplemented with 60 μ g/mL L-canavanine sulfate (Sigma).

2.2. Strains

All *S. cerevisiae* strains used in this study (Table 1) are isogenic with DFS188 (*MATa ura3-52 leu2-3, 112 lys2 his3 arg8::hisG; p⁺*) a derivative of D273-10B, except EAS738 and EAS736, which are derived from DFS160 [18]. The *rev1*- Δ , *rev3*- Δ , and *rev7*- Δ strains were constructed by one-step gene transplacement of the wild-type gene with the *kanMX* marker using standard

methods [19]. The GT+2 respiring microsatellite reporter (EAS736) was constructed as described previously except that the frameshift mutation results in translation in the +2 frame [20]. To introduce the mitochondrial reporter constructs by cytoduction, the *rev1-Δ*, *rev3-Δ*, and *rev7-Δ* strains were first made ρ^0 by treatment with ethidium bromide as described previously [21]. These strains were then mated to the DFS160 reporter constructs EAS738 (*arg8^m::(GT)₁₆+1*) and EAS736 (*arg8^m::(GT)₁₆+2*). Haploid cytoductants with the DFS188 nuclear background and the mitochondrial reporter were selected on the synthetic glycerol media lacking adenine and screened for a Lys⁻ phenotype on synthetic dextrose lacking lysine.

2.3. Survival after UV exposure

Average percent survival was determined for each experiment by calculating the number of viable cells after UV exposure. The percent survival within each strain was comparable for the different experiments, therefore the average percent survival is only shown in Table 2. The survival of the *rev* deficient strains at 50 J/m² (Mineralight lamp shortwave UV 254nm, UVP) is similar to previously published results [4,5].

2.4. UV-induced frequency of respiration loss

Determination of UV-induced frequency of respiration loss was performed as described previously [22]. Briefly, independent colonies were isolated on YPG and inoculated into YPG liquid medium. They were grown to saturation at 30°C and dilutions were plated on YPG + 0.1% dextrose media. These plates were exposed to a fluence of 50 J/m² UV-C and incubated in the dark at 30°C for 3 days. In all UV experiments, non-irradiated control plates were also incubated in the dark at 30°C for the same length of time. All plates were scored for the percent respiration loss. For each experiment five or more independent cultures were used and each experiment was performed two or more times.

2.5. Determination of UV-induced mutation frequencies

To determine UV-induced mitochondrial DNA (mtDNA) point mutations, independent colonies were isolated on YPG, inoculated into YPG liquid medium, and grown to saturation at 30°C. Appropriate dilutions were plated on YPG and YG + 4 g/L erythromycin. These plates were exposed to a fluence of 50 J/m² UV-C and incubated in the dark at 30°C for 7 days. To examine the UV sensitivity of the erythromycin resistant colonies, 15 independent colonies were inoculated into YPG liquid medium and grown to saturation at 30°C. Appropriate dilutions were plated on YPG and exposed to a fluence of 50 J/m² UV-C and incubated in the dark at 30°C for 3 days.

To determine the frequency of frameshifts in microsatellite tracts using the GT+1 and GT+2 reporters described above, independent colonies were isolated on YPD, inoculated into YPD liquid medium, and grown to saturation at 30°C. Appropriate dilutions were plated on YPD and synthetic dextrose media lacking arginine (SD-Arg). These plates were exposed to a fluence of 50 J/m² UV-C and incubated in the dark at 30°C for 7 days.

To determine the frequency of UV-induced nuclear mutations, independent colonies were isolated on synthetic dextrose media lacking arginine (SD-Arg) and inoculated into SD-Arg liquid medium. Cultures were grown to saturation at 30°C and dilutions were plated on SD-Arg and SD-Arg containing 60 μg/ml canavanine. The plates were exposed to a fluence of 50 J/m² UV-C and incubated in the dark at 30°C for 3 days.

The experiments were performed three or more times and each experiment consisted of five or more replicates. The number of new mutations per 10⁷ survivors was calculated by determining the frequency of mutations per 10⁷ cells from both UV-exposed and non-irradiated

control cultures. The preexisting mutations, as determined by the unirradiated control, were subtracted from the experimental values.

2.6. Fluctuation analysis to determine spontaneous mutation rates

The rates of spontaneous mtDNA point mutations were measured by erythromycin resistance (E^R) as described previously [23]. Briefly, independent colonies were isolated on YPG and inoculated into YPG liquid medium. Cultures were grown to saturation at 30°C and dilutions were plated on YPG and YG + 4 g/L erythromycin. The plates were incubated for 7 days at 30°C. The rates of E^R were determined using the method of the median [24].

For the determination of nuclear mutation rates, independent colonies were isolated on synthetic dextrose medium lacking arginine (SD-Arg) and inoculated into SD-Arg liquid medium. Cultures were grown to saturation at 30°C and dilutions were plated on SD-Arg and SD-Arg containing 60 µg/ml canavanine. The plates were incubated at 30°C for 3 days. The rates of nuclear point mutations as measured by canavanine resistance (Can^R) were determined using the method of the median [24]. Each experiment consisted of 20 independent cultures and the rates are given as an average of two or more independent experiments.

2.7. Sequencing of erythromycin resistant colonies

The spectrum of mtDNA point mutations was analyzed by sequencing a 200 bp region of the 21S rRNA gene within the mitochondrial genome. PCR was performed on E^R colonies derived from independent cultures using primers 5'-GGTAAATAGCAGCCTTATTATG-3' and 5'-CGATCTATCTAATTACAGTAAAGC-3' to amplify the region from 1797 to 1995 and primers 5'-CTATGTTTGCCACCTCGATGTC-3' and 5'-CAATAGATACACCATGGGTTGATTC-3' to amplify the region from 3895 to 4107. The amplified fragments were sequenced using primer: 5'-GAGGTCCCGCATGAATGACG-3' for region 1797 to 1995 and 5'-CTATGTTTGCCACCTCGATGTC-3' for region 3895 to 4107.

2.8. Statistical analyses

All statistical analysis was performed with InStat 3 for Macintosh (GraphPad Software, Inc., San Diego, CA). Unpaired t-tests were used to calculate a two-tailed p value in the comparison of average rates or frequencies. Comparison of the mutational spectra was performed using chi-square analysis.

3. Results

3.1. Deletion of *rev1*, *rev3*, or *rev7* reduces UV-induced respiration loss

Yeast cells in which mitochondrial function has been compromised are respiratory deficient. Such strains can be identified by their failure to utilize a non-fermentable carbon source for growth. Mutations in mtDNA, including point mutations, large-scale rearrangements or deletions, and the complete loss of mtDNA, have been demonstrated to result in respiratory-deficient yeast strains. To determine whether *rev1*, *rev3*, or *rev7* mutants affected respiration capacity, we measured the frequency of respiration loss after exposure to UV light. Consistent with a role for these proteins in error-prone recovery, deletion of *rev1*, *rev3*, or *rev7* significantly reduced the respiration loss phenotype seen in the wild-type (Table 2). These results suggest that Rev1p and Pol ζ contribute to general mitochondrial genome instability, however, the nature of the mutations that confer respiration loss in individual cells is not known, and is unlikely to simply reflect an accumulation of point mutations [20]. We therefore, examined the effect of loss of Rev1p, Rev3p and Rev7p on specific types of mitochondrial mutations.

3.2. Deletion of *rev1*, *rev3*, or *rev7* suppresses spontaneous and UV-induced mitochondrial microsatellite instability

Rev1p and Pol ζ are responsible for the majority of UV-induced nuclear frameshift mutations [25]. Pol ζ is also responsible for essentially all the spontaneous nuclear frameshift mutations [25]. Therefore, we analyzed the effect of deleting Pol ζ and Rev1p on mitochondrial frameshifts in microsatellite tracts. In these reporters, the *ARG8^m* gene containing a poly GT tract 9 base pairs down stream of its start is inserted upstream of the intact *COX2* gene, with its expression driven by the *COX2* promoter (Fig. 1). Insertions or deletions within this microsatellite that shift the *ARG8^m* gene into the correct reading frame will result in cells that are phenotypically Arg⁺. Two different microsatellite reporters were used, since reversion to wildtype will require different types of mutations in each reporter [23]. It is important to note that the poly GT tracts in these reporters are flanked by 4 pyrimidine base pairs; therefore TLS of pyrimidine dimers upstream of the repetitive tract may cause frameshifts within the tract.

Using these reporters, we have determined that deletion of the *rev* genes causes a reduction of both spontaneous and UV-induced microsatellite instability (Table 3). However, UV induction of these frameshifts is still observed in these strains, indicating that another pathway can generate these types of mutations.

3.3. Loss of Pol ζ results in an increase in spontaneous mitochondrial DNA point mutations

Erythromycin is an inhibitor of mitochondrial, but not cytoplasmic, translation. A mutation in either of two specific regions within the mitochondrially-encoded 21S rRNA gene has been shown to confer erythromycin resistance (*E^R*) [26–28]. There are no reported nuclear mutations in yeast that result in *E^R*; therefore, the frequency of *E^R* is often used to estimate the frequency of mitochondrial point mutation.

To determine whether deletion of *rev1*, *rev3*, or *rev7* had an effect on spontaneous mtDNA point mutations, we performed fluctuation analysis to measure the rate of spontaneous *E^R*. This assay revealed that the *rev3*- Δ and *rev7*- Δ strains had a significantly greater rate of spontaneous mtDNA point mutations than the wild-type strain ($p = 0.035$ and 0.029 , respectively), while the *rev1*- Δ strain showed no significant difference from wildtype (Fig. 2A).

3.4. Loss of Rev1p or Pol ζ results in increased frequencies of UV-induced mitochondrial erythromycin resistance

The increased spontaneous mutagenesis in the *rev3*- Δ and *rev7*- Δ strains was unexpected, as the strains are reported to display a decrease in spontaneous nuclear mutations. To determine whether such mutations are induced by UV exposure, we measured the frequency in which UV-induced *E^R* arose in *rev1*, *rev3*, and *rev7* deletion strains. Surprisingly, the frequency of mtDNA point mutations per survivor increased 20-fold over the wild-type, to approximately 1000 new mutations per 10^7 survivors (Fig. 2B). This result argues against a role for these proteins in the generation of UV-induced point mutations. Instead it suggests that these proteins maybe responsible for error-free bypass across these lesions. Furthermore, this provides evidence for a more error-prone mechanism that exists to cope with this damage when Rev1p and Pol ζ are not present. We considered the possibility that *E^R* cells were more resistant to UV light and preferentially survived the treatment. Therefore, independently isolated *E^R* mutants derived from the wild-type strain were tested for their sensitivity to killing by UV light exposure. These strains displayed an average of 60.6% viability in response to UV light, as compared to 70.5% for the original wild-type strain when exposed to the same fluence. We conclude that the *E^R* mutants are not more resistant to UV light, in contrast, our results demonstrate that they are significantly more sensitive ($p = 0.038$).

3.5. Identification of mutations giving rise to erythromycin resistance and analysis of mutations in the *rev1*, *rev3*, and *rev7* deletion strains

It was previously reported that a mutation at either of two locations within the 21S rRNA gene leads to E^R [26–28]. To determine the spectrum of mutations in spontaneous and UV-induced E^R mutants, we amplified the sequences flanking the locations of the previously published E^R mutations and sequenced across their length. The spontaneous E^R mutations identified in the wild-type strain include base substitutions at positions 1950, 1951, 1952, and a 1 base G insertion in the sequence of Gs at 1949 and 1950, which is similar to that previously reported by Vanderstraeten *et al.* [29].

Comparison of the spontaneous and UV-induced spectra of mutations within the wild-type, *rev1*-Δ, *rev3*-Δ, and *rev7*-Δ strains indicates that there is a significant increase in the proportion of substitutions at A residues after UV exposure ($p = 0.039$) (Table 4). Ninety-five percent of the UV-induced E^R mutations arise at position 1951, consistent with misinsertions opposite the 3' most T of a possible UV-induced pyrimidine dimer. Furthermore, comparison of the spectra of spontaneous and UV-induced mutations in the *rev1*-Δ, *rev3*-Δ, and *rev7*-Δ strains relative to the wildtype indicates that there is a significant difference only in the *rev1*-Δ strain. The spectrum of spontaneous mitochondrial point mutations in the *rev1*-Δ strain consists of 35% A → T transversions at position 1951, which is similar to the 33% observed in the wild-type strain. However following UV exposure, A → T transversions significantly increase to 76% in the *rev1*-Δ strain ($p = 0.012$) while there is no significant increase in the wild-type strain. These data indicate that in the mitochondrial compartment, the mechanism of UV recovery in the absence of Rev1p causes a shift in the mutational spectra that results primarily in A → T transversions.

3.6. Verification of reduced nuclear mutagenesis in the *rev1*, *rev3*, and *rev7* deletion strains

In order to ensure that the increased point mutation accumulation in the *rev* mutant strains is mitochondrial specific, we investigated the nuclear point mutation as measured by canavanine resistance (Can^R). Canavanine is a lethal arginine analog that is taken up by the arginine permease, encoded by the *CAN1* gene. Cells that have the ability to produce their own arginine and acquire a mutation in *CAN1* gene can grow on medium lacking arginine and containing canavanine.

Using this drug, we can estimate spontaneous nuclear point mutation accumulation in the *rev* deletion strains. This assay indicates that while the *rev1*-Δ strain has a nuclear point mutation rate that is not significantly different from the wild-type strain, deletion of *rev3* or *rev7* results in a small, but significant decrease in spontaneous nuclear point mutations ($p = 0.013$ and 0.042 , respectively) (Fig. 3A).

Additionally, we observed a significant decrease in UV-induced nuclear DNA point mutations in all three *rev* mutant strains as compared to the wild-type strain (Fig. 3B). Since the strains used for these studies were derived from those used to measure mtDNA point mutations, this important control demonstrates that the strains we are examining behave as expected with respect to nuclear point mutations.

4. Discussion

The significance of mitochondrial mutagenesis is underscored by the accumulation of mtDNA mutations, rearrangements, and deletions associated with aging and aging-related disorders [30,31]. Evidence for UV-induced mtDNA point mutations was first published in 1979 [32], however, the protein components and pathways responsible for this mutagenesis was unknown. We now know that translesion polymerases are responsible for nuclear UV-induced

mutagenesis, however, the mechanisms by which mitochondrial mutations are generated remain unknown.

Pol ζ and Rev1p are responsible for error-prone translesion synthesis in the nucleus [1]. Together these enzymes are responsible for both UV-induced and spontaneous mutagenesis [1]. Recently fusions of the N-termini of Rev1p and the proteins that comprise Pol ζ (Rev3p and Rev7p) with GFP suggested localization to the mitochondrial compartment of *S. cerevisiae* [15].

We have completed a comprehensive analysis of the different types of mitochondrial mutations associated with the deletion of *rev1*, *rev3*, and *rev7*. Consistent with a previous report, analysis of mitochondrial microsatellite instability in *rev* mutant strains demonstrates that Rev1p and Pol ζ are responsible for the majority of spontaneous and UV-induced mitochondrial frameshifts [15]. Furthermore, our data also indicates that these proteins play a role in the generation of UV-induced respiration loss. In contrast, we have determined that Pol ζ is responsible for preventing spontaneous mitochondrial erythromycin resistant mutations. In addition, although deleting the translesion polymerases Pol ζ and Rev1p results in a decrease of some types of mutations, their loss causes a dramatic increase in UV-induced mtDNA point mutations as measured by erythromycin resistance. Since there are limited tools available to measure mtDNA point mutations, we are only able to observe the spectra of mutations at one locus. However, the range of substitutions which give rise to E^R in the 21S rRNA suggests that the observed increases are not limited to specific base substitutions. Regardless of its generality, this observation reveals the existence of an alternative mutagenic process. Furthermore, the spectra of mutations resulting in erythromycin resistance suggest that Rev1p is responsible for the spectrum produced in the wild-type strain. Although the increase in point mutations in the *rev* deficient strains may appear to be at odds with the decrease in respiration loss, we have previously observed that the rates of these types of mutations do not correlate well [20], suggesting that the mechanism by which they arise is different. Consistent with our previous results, we believe the non-respiring cells are unlikely to result primarily from an accumulation of point mutations.

Since converting DNA lesions into mutations is an active process, resulting from mechanisms of damage tolerance, our data indicate that there is another pathway that is more mutagenic than Rev1p and Pol ζ , which is responsible for generating mitochondrial mutations in their absence. Since these phenotypes are only observed for mtDNA and there is no apparent increase in nuclear mutations, we hypothesize that this alternative pathway is mitochondrial specific. The only other known polymerase in the mitochondrial compartment is Pol γ , which is responsible for replication of the mitochondrial genome. Therefore, we speculate that in the absence of TLS by Rev1p and Pol ζ , a modification of Pol γ may be responsible for creating some of these mutations in efforts to bypass lesions that block the replication fork. The Pol32 subunit of the yeast nuclear replicative polymerase, Pol δ , is involved in mutagenesis [33–35]. Therefore, we are interested in investigating whether Pol32 or another protein may associate with Pol γ to allow bypass of UV-induced lesions in the absence of Rev1p and Pol ζ .

The mechanism involved in UV-induced mtDNA damage tolerance in yeast and higher eukaryotes may be conserved considering the conservation of these polymerases among species. Recently therapeutic inhibition of Pol ζ has been proposed for the reduction of mutagenesis in cancer cells [36]. Our results suggest that such inhibition may adversely impact mtDNA.

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Fig. 1.

Effect of deleting *REV1*, *REV3*, and *REV7* on mitochondrial microsatellite instability in respiring yeast. The reporters for measuring mitochondrial microsatellite instability are shown. *ARG8^m* is a derivative of the nuclear *ARG8* gene, altered to display the codon usage of a mitochondrial gene. This gene is expressed using the *COX2* transcriptional and translational signals, and has been inserted 5' of the intact *COX2* gene in the mitochondrial genome. The polyGT sequences have been inserted 9 bp after the translational start. The GT repeats are 32 bp in each reporter but the flanking sequences have been altered to generate a (A) +1 or (B) +2 frameshift.

Fig. 2. Effect of deleting *REV1*, *REV3*, and *REV7* on mitochondrial DNA point mutations as measured by erythromycin resistance. (A) The spontaneous rate of mtDNA point mutations was determined by fluctuation analysis as described in Materials and Methods. The average rates from two or more independent experiments are plotted. Error bars indicate the SEM. Statistical analysis using unpaired two-tailed t-tests indicate that *rev3-Δ* and *rev7-Δ* have a significantly higher rate of mtDNA point mutations than wildtype ($p = 0.035$ and 0.029 , respectively). (B)

The UV-induced frequency of mtDNA point mutations as measured by erythromycin resistance. Deletion of *REV1*, *REV3*, or *REV7* results in a significantly higher frequency of erythromycin resistant colonies per survivor following exposure to 50 J/m² UV-C ($p < 0.001$ in all cases). The average frequencies from three independent experiments are plotted. Error bars indicate the SEM.

Fig. 3.

Nuclear point mutations as measured by canavanine resistance. (A) The spontaneous rate of canavanine resistance was determined by fluctuation analysis as described in Materials and Methods. The average rates from two or more independent experiments are plotted. Error bars indicated the SEM. Statistical analysis indicates that although *rev1-Δ* displays no significant difference from wildtype, *rev3-Δ* and *rev7-Δ* show a slight but significant decrease in nuclear point mutations ($p = 0.013$ and 0.042 , respectively). (B) The frequency of nuclear point

mutations per survivor after exposure to 50 J/m² UV-C. The average frequency of 2–3 independent experiments is plotted. Error bars indicate the SEM. Statistical analysis reveals that the frequency of canavanine resistance in the *rev1*-Δ, *rev3*-Δ, and *rev7*-Δ strains is significantly lower than that of the wild-type strain ($p = < 0.001$ in all cases).

Table 1

Strains used in this study

Strain	Relevant Nuclear Genotype	Relevant Mitochondrial Genotype	Reference
DFS188	<i>MATa ura3-52 leu2-3, 112 lys2 his3 arg8::hisG</i>	ρ^+	[23]
DFS160	<i>MATa ade2-101 leu2Δ ura3-52 arg8-Δ::URA3</i>	ρ^0	[18]
TF236	<i>ino1::HIS3 arg8::hisG pet9 (op1) ura3-52 lys2 cox3::arg8m-1</i>	ρ^+	[37]
EAS738	<i>MATa ade2-101 leu2Δ ura3-52 arg8-Δ::URA3</i>	$\rho^+ \arg8^{gm::(\text{GT})16^{+1}}$	[20]
EAS744	<i>MATa ura3-52 leu2-3, 112 lys2 his3 arg8::hisG</i>	$\rho^+ \arg8^{gm::(\text{GT})16^{+1}}$	[20]
EAS736	<i>MATa ade2-101 leu2Δ ura3-52 arg8-Δ::URA3</i>	$\rho^+ \arg8^{gm::(\text{GT})16^{+2}}$	This Study
EAS741	<i>MATa ura3-52 leu2-3, 112 lys2 his3 arg8::hisG</i>	$\rho^+ \arg8^{gm::(\text{GT})16^{+2}}$	This Study
NPY054	<i>MATa ura3-52 leu2-3, 112 lys2 his3 arg8::hisG ARG8</i>	ρ^+	[38]
LKY118	DFS188 <i>rev1-Δ::kanMX</i>	ρ^+	This Study
LKY185	DFS188 <i>rev1-Δ::kanMX ARG8</i>	ρ^+	This Study
LKY132	DFS188 <i>rev1-Δ::kanMX</i>	$\rho^+ \arg8^{gm::(\text{GT})16^{+1}}$	This Study
LKY131	DFS188 <i>rev1-Δ::kanMX</i>	$\rho^+ \arg8^{gm::(\text{GT})16^{+2}}$	This Study
EAS798	DFS188 <i>rev3-Δ::kanMX</i>	ρ^+	This Study
LKY125	DFS188 <i>rev3-Δ::kanMX ARG8</i>	ρ^+	This Study
LKY151	DFS188 <i>rev3-Δ::kanMX</i>	$\rho^+ \arg8^{gm::(\text{GT})16^{+1}}$	This Study
LKY150	DFS188 <i>rev3-Δ::kanMX</i>	$\rho^+ \arg8^{gm::(\text{GT})16^{+2}}$	This Study
LKY119	DFS188 <i>rev7-Δ::kanMX</i>	ρ^+	This Study
LKY186	DFS188 <i>rev7-Δ::kanMX ARG8</i>	ρ^+	This Study
LKY133	DFS188 <i>rev7-Δ::kanMX</i>	$\rho^+ \arg8^{gm::(\text{GT})16^{+1}}$	This Study
LKY149	DFS188 <i>rev7-Δ::kanMX</i>	$\rho^+ \arg8^{gm::(\text{GT})16^{+2}}$	This Study

Table 2

UV-Induced Respiration Loss

Strains	Survival	Spontaneous Respiration Loss	UV-Induced Respiration Loss
Wild-Type	70.5	0.7	6.0
<i>rev1</i> -Δ	1.0	1.3	1.2
<i>rev3</i> -Δ	1.6	0.5	< 0.1
<i>rev7</i> -Δ	1.1	1.3	< 0.1

* Values for survival and respiration loss are indicated as percents.

** Decreases in UV-induced respiration loss of the *rev* deficient strains are significant as determined by unpaired t-tests ($p < 0.00005$).

Table 3

Mitochondrial Microsatellite Instability

Strains	GT + 1				GT + 2			
	Spontaneous		UV-Induced		Spontaneous		UV-Induced	
	Average Frequency of Arg ⁺ Frameshifts per 10 ⁷ Cells	Fold Decrease	Average Frequency of Arg ⁺ Frameshifts per 10 ⁷ Survivors	Fold Decrease	Average Frequency of Arg ⁺ Frameshifts per 10 ⁷ Cells	Fold Decrease	Average Frequency of Arg ⁺ Frameshifts per 10 ⁷ Survivors	Fold Decrease
Wild-Type	48.6	-	1026.9	-	68.8	-	1254.4	-
<i>rev1-Δ</i>	0.1	486.0	2.9	354.1	5.5	12.5	44.7	28.1
<i>rev3-Δ</i>	3.9	12.5	45.9	22.4	24.1	2.9	54.1	23.2
<i>rev7-Δ</i>	5.9	8.2	50.3	20.4	1.3	52.9	15.1	83.1

* All decreases relative to wildtype are statistically significant as determined by unpaired t-tests (p < 0.05).

Table 4

The Spectra of Erythromycin Resistant Mutations

Strains	% with nucleotide substitutions (no.)									
	1950			1951			1952			3993
	G→T	G→A	A→T	A→G	A→C	A→T	A→G	G ins	C→G	
Wild-type	5.6 (2)	11.1 (4)	33.3 (12)	27.8 (10)	2.7 (1)	5.6 (2)	5.6 (2)	8.3 (3)	0	
rev1-Δ	0	5.0 (2)	35.0 (14)	32.5 (13)	10.0 (4)	0	10.0 (4)	2.5 (1)	5.0 (2)	
rev3-Δ	0	20.8 (5)	37.5 (9)	33.3 (8)	0	4.2 (1)	0	4.2 (1)	0	
rev7-Δ	0	0	28.6 (4)	50.0 (7)	7.1 (1)	7.1 (1)	7.1 (1)	0	0	
Wild-type	0	0	35.0 (7)	55.0 (11)	5.0 (1)	0	5.0 (1)	0	0	
rev1-Δ	0	0	75.9 (22)	13.8 (4)	6.9 (2)	3.4 (1)	0	0	0	
rev3-Δ	0	0	53.8 (14)	26.9 (7)	7.7 (2)	0	7.7 (2)	3.8 (1)	0	
rev7-Δ	0	0	20.0 (1)	60.0 (3)	0	0	0	20.0 (1)	0	

* A total of 194 independent erythromycin resistant colonies were sequenced. Only two mutations were found at position 3993. These were: rev1-Δ C→G (2).